

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: TREMBLAY, Réjean et al.
Serial number: 10/521,868
Filing date: August 30, 2005
For: PROCESS FOR INCREASING THE YIELD OF LIPID AND
OMEGA-3 FATTY ACID IN SEAWEED CULTURE
Art Unit: 1651
Examiner: MARX, Irene
Agent: David S. Resnick

DECLARATION UNDER 37 C.F.R. SEC. 1.132

I, Réjean Tremblay, do hereby declare and state as follows:

1. I received the degrees of Bachelor of biology from Université du Québec à Rimouski in 1989, Master of oceanography from Université du Québec à Rimouski in 1997, and Doctor of Philosophy (Biology) from Université Laval in 1997.
2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.
3. I have been a Professor in Aquaculture at l'Institut des sciences de la mer de Rimouski since october 2003
4. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.
5. I am an inventor in the present application; I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. 10/521,868, entitled "PROCESS FOR INCREASING THE YIELD OF LIPID AND OMEGA-3 FATTY ACID IN SEAWEED CULTURE", including the claims.

6. I have also read and understood the latest Official Action from the PTO dated August 17, 2007. In this Office Action, the Examiner requested election of a single species.
7. The following experiments were performed from November 2007 to February 2008, under my supervision, to demonstrate that another member of the diatomaceous algae family (*Thalassiosira pseudonana*), when stimulated following the teachings recited in U.S. Patent Application Serial No. 10/521,868, will produce polyunsaturated fatty acids (PUFAs).

Enrichment in polyunsaturated fatty acids following stress of silicate

Thalassiosira pseudonana was cultured in a semi-continuous system of 170 liters. 4 X 200 liters of extracted algae were submitted to a stress of silicate for 7 days. For lipid analysis, 50 X 10⁶ cells were used to evaluate the lipid content before and after the stress of silicate was applied. Classes of lipids were determined by liquid chromatography. Fatty acids were identified by comparing the retention time obtained with that of a known standard (FAME, composed of 37 elements, PUFA-3, BAME, Supelco Bellefonte, PA). Quantification was accomplished with a known standard. The results are presented in the following table as percentage (%) of total lipid:

		Before stress	After 4 days of stress
T. <i>pseudonana</i>	20:5n-3 (EPA)	26.5 ± 1.2	39.5 ± 5.4
	Total of polyunsaturated fatty acids	50.6 ± 5.8	65.5 ± 7.6

Conclusion: As demonstrated, an enrichment of PUFAs was observed in cultures of *T. pseudonana* after 4 days of induced stress. *T. pseudonana* is rich in eicosapentanoic acid (EPA, 20:5n-3). The content of EPA was also increased following 4 days of stress with silicate.

8. The results presented above and produced according to the teachings of the present application clearly indicate that another member of the diatomaceous

algae family (*Thalassiosira pseudonana*), when stimulated following the teachings recited in U.S. Patent Application Serial No. 10/521,868, will produce polyunsaturated fatty acids.

9. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed



Date:

4 February 2008

Réjean Tremblay